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Why Yellow-Poplar Seeds Have Low Viability

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Yellow-poplar (*Liriodendron tulipifera* L.) trees normally produce a small percentage of viable seeds. Usually only about 10 percent and seldom more than 30 percent of the samaras contain any viable seed. When based on the number of potential seeds, viability is rarely more than 5 percent. However, the number of seeds with embryos present varies greatly, not only from tree to tree but also from year to year *(7, 10, 11, 14, 16)*[1].

We wanted to know why yellow-poplar trees produced seeds of low viability. We thought the answer might be found in the developmental processes leading to the formation of seeds. So we observed the processes of flower development, pollination, fertilization, and embryo and endosperm development. We looked for irregularities in all developmental processes and in chromosomal behavior, for evidences of maternal and bipaternal origins of embryos, and for deterioration of developing embryos since this had been previously postulated *(7)*. And, since it has been reported that self-pollination produces lower percentages of seeds with embryos than does open or controlled cross-pollination *(6, 17)*, we looked for differences in the compatibility of pollen from the same tree and among trees.

Our studies show that yellow-poplar trees produce seeds of low viability because of ineffective pollination which results in a small number of fertilized egg cells. Most pollen is incompatible with styles of the same tree so that self-pollination is mostly ineffective. And cross-pollination is effective only when the trees are compatible. Some cross-pollinations are no more effective than self-pollination, while other cross-pollinations may result in most of the seeds being viable. Although chances for fertilization are better if the flowers are cross-pollinated, relatively little crossing occurs because insect pollination among trees is relatively inefficient. Thus, during the short receptive period of the stigmas, 12 to 24 daylight hours, few of the approximately 80 stigmas of each flower receive compatible pollen. Also, rain and other weather conditions that reduce the activity of insects cause a reduction in the percentage of viable seeds. Therefore, the percentage of viable seeds is determined by a number of chance occurrences that result in effective pollination of some stigmas.

We found no irregularities in developmental processes that could result in nonviable seeds. The development of pollen,

[1]Numbers in parentheses refer to Literature Cited, page 15.

ovules, egg sacs, and eggs were normal and regular. We found no chromosomal irregularities which would interfere with fertilization or stop the formation of embryos and endosperms. There was no evidence that large numbers of embryos deteriorated before the seeds matured. Apparently, if flowers are pollinated with compatible pollen when the stigmas are receptive, then fertilization follows and embryos and endosperms develop inside the ovules and result in normal, viable seeds.

We can increase the production of viable seeds in seed-production areas and seed orchards by crossing compatible trees or by introducing compatible pollen. These results also mean that yellow-poplar trees are not freely interbreeding and that genetic variability is maintained by limited crossings among closely related trees and limited self-fertilization. Because of this, seeds for nurseries should be collected from a number of trees located more than 1 mile apart. These results also indicate that it may be difficult to breed yellow-poplar for desirable characteristics. If genes for a desirable characteristic are linked with the genes for incompatibility, certain gametes will not occur in the offspring and the genetic ratios will be distorted.

MATERIALS AND METHODS

Periodic collections were made of flower buds of different sizes, unopened and opened flowers, and of fruits from trees growing in southern Illinois. Collections were made about every 2 weeks from mid-April until about the middle of July of 1958, and again in 1959 from about the middle of April until the middle of August. Samaras for dissections and for germination studies were collected shortly before shedding during both growing seasons.

Materials for microscopic examination of sections were, after dissection, killed and fixed in either F.A.A. or CRAF, and then aspirated. CRAF treated materials were stored in 70 percent ethyl alcohol whenever necessary. The usual ethyl alcohol-tertiary butyl alcohol series was used for infiltration, and the specimens were imbedded in paraffin. Serial and longitudinal sections were cut at from 10 to 14 microns and were stained in either safranin 0 and fast green FCF, or in Delafield's haematoxylin *(8, 13)*.

Stamens for the study of developing pollen grains were killed and fixed in absolute alcohol-glacial acetic acid (2:1), aspirated, and after 2 weeks transferred to 80 percent ethyl alcohol for storage. Acetocarmine smears were prepared for studies of chromosomes and of meiosis in developing pollen grains. Stamens were also studied after sectioning, following the methods outlined previously.

Transverse sections of young cones were also made with a sliding microtome in order to study the development of embryos and endosperm.

In 1958, 1959 , and 1960 a small number of controlled pollinations were made among three groups of trees. These pollinations provided material for studying developmental processes in self-, cross-, and un-pollinated flowers. They also provided additional evidence for differences in compatibility among widely separated trees. The trees in each group were growing in open areas, had large crowns, produced many flowers, and were not competing with other large trees. The three trees in Group I were within 200 feet of each other and were among eight other yellow-poplar trees. The two trees in Group II were adjacent, were 21 miles from Group I, and were more than a mile from any other yellow-poplar trees. The two trees in Group III were about 60 feet apart, were 18 miles from Group I, 9 miles from Group II, and were among 10 other yellow-poplar trees. Some flowers were emasculated, not pollinated, and bagged; others were emasculated, self-pollinated, and bagged; and some were emasculated, cross-pollinated, and bagged. Cones from these flowers and from open-pollinated flowers were examined in May, June, and August. Some of the samaras were studied

after hand dissection and others after microtome sectioning.

The number of seeds filled with endosperm, and presumably containing embryos, was determined by sectioning a sample of the samaras. Some of the samaras were used for germination studies in a greenhouse. However, germination studies were found to be unreliable and inconvenient because the rate of germination was influenced by storage conditions and 3 years were usually required for all of the viable seed to germinate.

In 1958, 1959, and 1960 records were made of the development of flower buds, of flowering, and of natural pollination. Records were also kept of the various insects found in and around the flowers and of weather conditions during the time of flowering.

RESULTS

General Structure of the Flower

Mature yellow-poplar flowers are bisexual, symmetrical, solitary, large, and showy (fig. 1). The calyx consists of three sepals which become reflexed. The corolla, composed of two rows of three petals each, is bell-shaped when open. Nectaries are located on bright orange spots near the base of the petals. These bright-colored spots and the associated large droplets of sweet nectar attract many insects. The stamens, totaling 20 to 30 per flower, are separate and have elongated anthers on short filaments. They dehisce longitudinally beginning at the base. The lines of suture of the anthers are directed away from the pistils and this tends to reduce self-pollination. At maturity the pollen grains are covered with a mucilage. When the anthers open the mucilage and pollen form sticky masses which adhere to all parts of the flower and to insects. It is doubtful if wind ever carries these sticky pollen masses very far and certainly rarely causes cross-pollination. The flat scale-like pistils are separate and are spirally arranged on a conical stalk to form an elongated cone. The single stigma at the tip of each pistil is hairy and reflexed. Insects walking over the cone undoubtedly brush against some of the hairy stigmas. At maturity the stigmas are coated with a milky-white mucilage which traps masses of pollen grains from the bodies of insects.

FIGURE 1. — *Yellow-poplar flowers are large and showy. On the second day of flowering, as shown in this drawing, the petals have moved away from the stamens and the anthers have split open to release the pollen on the sides directed away from the centrally located pistils. The dark areas at the base of the petals are bright orange and indicate the location of nectaries. At this stage, the stigmas are black and are not receptive to pollen.*

The flower buds are formed by October, but are not readily recognized by their external appearance during the winter months. As with the vegetative buds, the bud scales of the flower bud are modified stipules, and thus the shapes of the two kinds of buds in early stages are externally indistinguishable. After the initial spurt of bud growth about the middle of April, the comparatively rapid enlargement of the flower parts causes the flower buds to become more elliptical than the vegetative buds.

The pistils are formed in the flower buds in the fall and overwinter as small structures about 1 millimeter in length Each mature, fertile, pistil is a 2-seeded samara 2 to 5 centimeters long (fig. 2). The two seeds are basal, occur in a single cavity, and either or both seeds may be viable or empty. The lowermost 10 to 14 pistils are sterile and at maturity are persistent. When the cones mature in the fall the dehisced fertile samaras are loosely held in a basket-like structure formed by the sterile samaras. The loose, fertile samaras are disseminated when the branches are shaken by wind.

Development of Ovules, Embryo Sacs, and Egg Cells

We found the ovules enlarging and developing rapidly during the third and fourth weeks of April. During the first week of May the earliest flowers were open. During this period we followed the development of the ovules from small domes of cells with no apparent differentiation to mature ovules ready for fertilization. As the ovule develops the funiculus is elongated and the ovule is turned so the micropylar ends are directed upward toward the stigma (fig. 3). When the ovule is well developed, an obturator, which forms from the funiculus, has grown over the micropylar end of the ovule. Thus

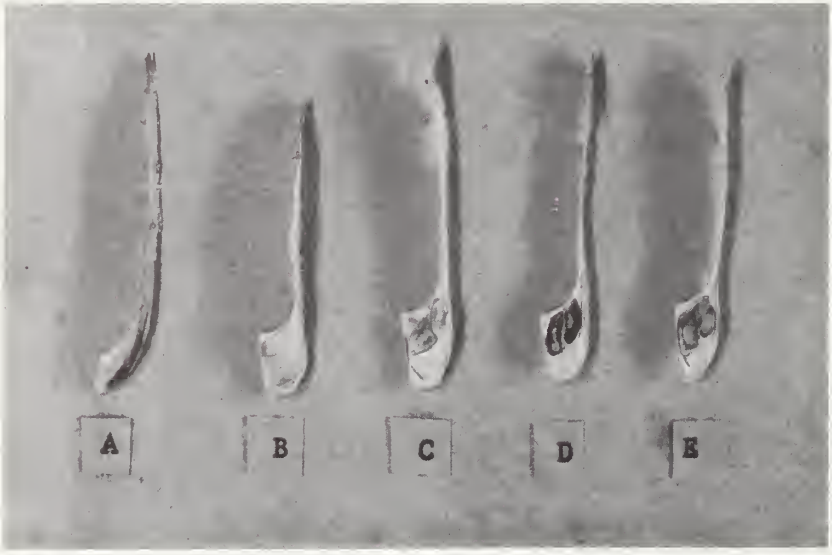

FIGURE 2.—*Each mature pistil is a samara. Sterile samaras* (A) *have no seed, occur at the base of the cone, and are persistent. Fertile samaras* (B, C, D, E) *contain two seeds each which form from ovules. Portions of the samaras* C, D, *and* E *have been removed to show the position of the seeds* (C), *two non-viable seeds* (D), *and two viable seeds* (E).

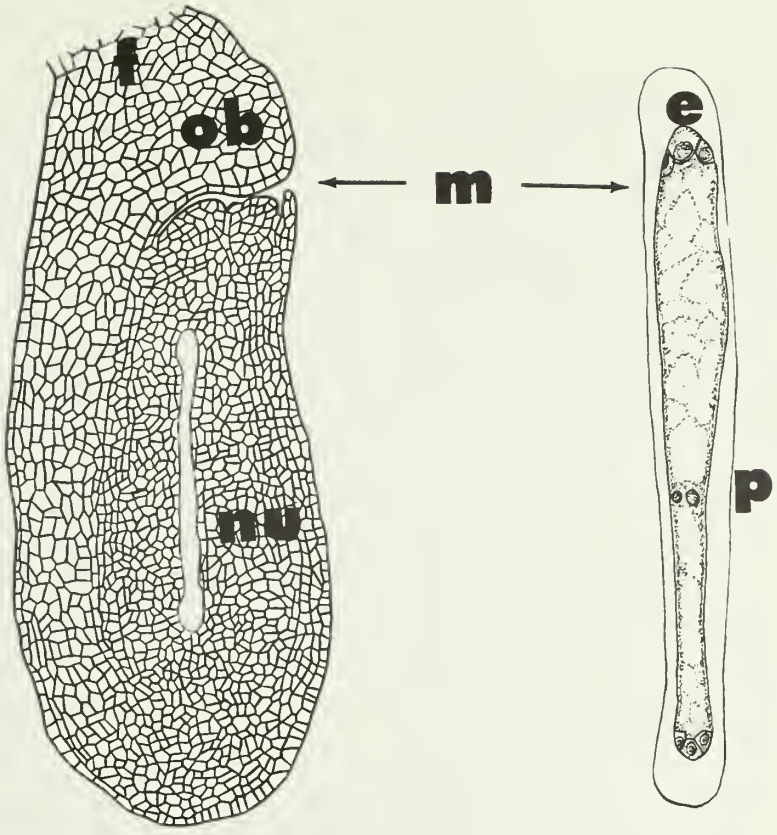

FIGURE 3. — *Drawing of a mature ovule at the time of pollination. Each fertile pistil contains two ovules which are attached by the funiculus* (f) *in the same positions as the seeds in the samaras of figure 2. The ovule is turned so the micropylar end* (m) *is directed upward toward the stigma. An obturator* (ob) *forms a kind of bridge so that pollen tubes can grow directly through the funiculus* (f), *the obturator* (ob), *and the nucellus* (nu) *to the elongated embryo sac which is embedded in the nucellus.*

FIGURE 4. — *Enlarged drawing of a mature embryo sac as shown in figure 3. The large egg cell* (e) *is at the micropylar end* (m). *During fertilization the egg cell unites with a sperm to form an embryo with cells containing 38 chromosomes. The two polar nuclei* (p) *soon fuse to form the fusion nucleus which unites with a sperm and forms the endosperm tissue with cells containing 57 chromosomes.*

pollen tubes can travel from the style into the funiculus and thence through the obturator, using it as a kind of bridge to reach the micropyle. This greatly reduces the distance the pollen tubes would otherwise have to travel to reach the embryo sac.

The embryo sac forms as the ovule develops. We followed the formation of the embryo sac from a single cell to maturity. The mature embryo sac is long and narrow with somewhat bulging ends (fig. 4). Three uninucleated cells occur at the micropylar end, two polar nuclei are centrally located, and three uninucleated cells are at the opposite end. The large cell at the micropylar end is the egg cell. The polar nuclei soon fuse, forming the fusion nucleus, which, during fertilization, unites with a sperm and forms the endosperm tissue. Cells of the developing endosperm are large and have large, dark-staining nuclei that are easily distinguished from the nucellus cells which surround the embryo sac.

The surrounding nucellar tissue appears "normal" except for one or two layers of cells immediately surrounding the embryo sac. The cells of this area appear collapsed and with solidly stained nuclei, conditions usually attributed to the effects of resorption of materials by the embryo sac.

All ovules of a flower are about the same size. The two ovules in each of the approximately 80 pistils in each flower mature at the same time although ovules in the upper pistils of some flowers appeared to be in a slightly younger stage of development than those in the lower pistils. All embryo sacs of a flower are mature and ready for fertilization when the flower opens. Thus, if many viable seeds are to be formed, the approximately 80 fertile pistils must be pollinated at about the same time and very soon after the flower opens.

Apparently all fertile pistils contain normal, mature embryo sacs, each with an egg and two polar nuclei. We found no evidence of the occurrence of irregular, poorly formed, or immature embryo sacs which could cause non-viable seed. The obturators are well developed and cover the micropylar openings so that continuous tissue is available for compatible pollen tubes to grow through to the embryo sacs. The stigmatic surfaces of all of the pistils showed numerous, well-developed, glandular hairs. There was no evidence of physical barriers to pollen-tube development.

Development of Pollen and Sperm Cells

Anthers form in the fall but pollen does not mature until the last week of April and the first week of May. Most of the pollen is in the binucleate stage when the anthers open. The small generative cell is surrounded by its own proportionately small amount of cytoplasm; the pollen tube nucleus is larger and its cytoplasm fills most of the pollen grain (fig. 5). When the flowers open, pollen grains from unopened and opened anthers are well developed and there are very few undersized, empty, or shriveled grains. Pollen from unopened anthers can be successfully used for cross-pollinations.

FIGURE 5. — *Drawing of a grain of pollen shortly before the time of shedding. The large tube nucleus* (t) *and its cytoplasm fill most of the grain. The small generative cell* (g) *divides during the growth of the pollen tube to form two sperms.*

The sperm cells usually form in the pollen tubes as they pass through the pistil. In naturally pollinated flowers only a few pollen tubes were found, and these were seen only between the cells of the nucellus. The three nuclei, (the pollen tube nucleus and the two sperm nuclei), were found and no abnormalities were observed. We expected to find, as in many plants, that many pollen tubes developed in the style, but that only one takes part in fertilization. The small number of pollen tubes in pistils of controlled pollinated flowers indicated that few pollen grains germinated on the stigma or most of the pollen tubes did not develop very far beyond the stigma. However, observations of stigmas several hours after pollination showed many germinating pollen grains. Therefore, it seems that pollen tubes from incompatible pollen do not grow very far beyond the stigma.

There was no evidence that low viability of pollen was responsible for the observed low viability of seeds.

Observations of Chromosomes

No chromosomal irregularities were noted in any of the preparations. Chromosomes were frequently observed during the formation of pollen and were observed once during the formation of an embryo sac. We found no evidence that chromosomal irregularities cause the low viability of seed.

Our observations confirm previous reports that yellow-poplar has 19 pairs of chromosomes *(12, 15)*. Ten counts were made during the formation of pollen in three different trees and one count was made in the prophase stage of the first meiotic division preceding the formation of female spores (fig. 6). To our knowledge this is the first time a chromosome count has been made during the formation of the female structures of yellow-poplar.

Our observations on mature pollen also indicate a lack of chromosomal irregularities. Practically all of the mature pollen observed within anthers was uniform in size and no empty or shriveled grains were

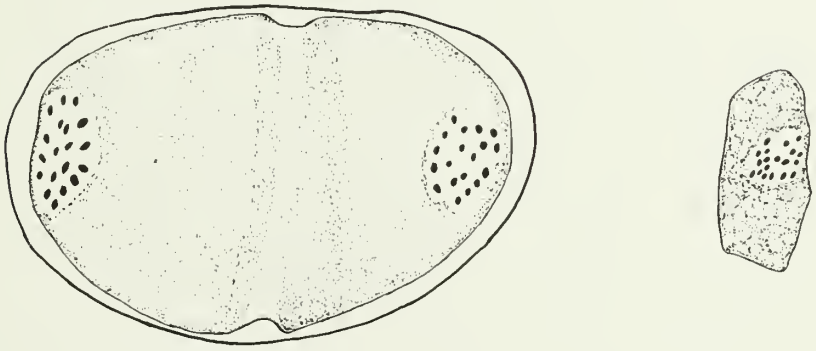

FIGURE 6.—(RIGHT) *Drawing of a female spore during a stage of cell division which shows the basic number of chromosomes to be 19. At this stage, the chromosomes appear as short, rod-like structures.* (LEFT) *Drawing of a developing pollen grain at a stage of cell division which shows the basic number of chromosomes to be 19. We observed no irregularities in either the number or the morphology of chromosomes.*

noted. If chromosomal irregularities occurred frequently enough to account for the observed low viability of seed, the effects should be reflected in the formation of pollen and we should have seen at least one abnormality among the many preparations.

Controlled Pollinations

We made a few controlled pollinations, and the results were essentially the same as those obtained by Carpenter and Guard (*2*) and by Wright (*17*). Controlled cross-pollinations usually resulted in higher percentages of filled seed than controlled self- and uncontrolled insect pollinations. And crosses among widely separated trees usually resulted in more filled seed than crosses among trees of the same stand.

Trees 4 and 5 (table 1) produced the least number of filled seed from insect pollinations because these two trees have low compatibility, they flower at different times, and they are more than a mile from other yellow-poplar trees. Crosses between these trees resulted in 3 to 6 percent filled seed while pollen from tree 6, 9 miles away, resulted in 13 to 15 percent filled seed. And, trees 4 and 5 were more compatible with trees 1 and 2, 21 miles away, than with each other. Also, tree 4 began flowering about 10 days before tree 5, which reduced the number of filled seed because the earliest flowers of tree 4 and the latest flowers of tree 5 were apparently self-pollinated.

Trees 3 and 7 seemed to be the most compatible. Crosses between these trees, which are 18 miles apart, resulted in 30 to 43 percent filled seed. Both of these trees were good producers of filled seed when controlled cross-pollinated, but produced only about 1 percent filled seed when controlled self-pollinated.

Apparently trees of the same stand are likely to be closely related and therefore have lower compatibilities than trees of different stands. For this reason widely separated trees are usually more compatible than trees of the same stand.

Natural Pollinations

If the percentage of viable seeds formed is to be high, compatible pollen must reach the stigmas during the period of receptivity. Conditions that influence the source of the pollen, the time of pollination, and the number of stigmas pollinated will influence the percentage of viable seeds. Our studies indicate that few stigmas received compatible pollen because the receptive period of the stigmas is short, because cross-pollination by insects is relatively inefficient, and because highly compatible trees are rarely adjacent.

We found the stigmas to be mature and receptive to pollen at least a day before the flowers normally open. And, although the pollen is functional several days before the flowers open, the anthers rarely dehisce until the second day of opening. On the first day of opening the petals separate near their tips forming an opening about ½ to 1 inch in diameter. The tip of the cone is in the center of this opening and insects can conveniently climb down to the nectaries on it. At this stage of flower development the stigmas are reflexed and coated with milky-white mucilage that may collect pollen from the insects. The anthers are closed and, since the sutured sides are closely appressed against the petals, there is little chance for insects to break the sutures. Insects leaving the flower either climb the pistils or the non-sutured sides of the stamens. Most flowers remain in this condition throughout the first day of opening. However, if the day is very windy or warm, the flower may open further. Many anthers begin to dehisce near their bases during the evening of the first day of opening. The flowers remain open during the night, but it is doubtful if much pollination occurs at night.

On the second day after opening the petals separate forming a bell-shaped flower; the stamens stand free between the pistil and the petals, and the anthers completely dehisce. Masses of pollen are held together by mucus, keeping the pollen from being blown from the flowers. The bases of the petals become covered with pollen and insects visiting the nectaries collect pollen on their bodies. Insects leaving the flowers most frequently climb the cone and thus self-pollinate some of the

Table 1. — *The percentages of filled seeds resulting from cross-pollinations, self-pollinations, and insect pollinations in 1958, 1959 and 1960*

Tree group	Female tree	Type of pollination[2]	Male tree	Percent[1] of seeds filled with endosperm by year		
				1958	1959	1960
I	1	cross	3	12.0	.....	.....
	1	cross	4	14.1	15.2	.....
	1	cross	7	.....	9.3	10.3
	1	self		0.0	.4	.....
	1	insect		7.7	7.5	2.6
	2	cross	1	6.1	.....	.....
	2	cross	5	14.3	.....	.....
	2	cross	7	19.0	.....	20.1
	2	self		0.0	.4	.....
	2	insect		4.5	3.8	1.0
	3	cross	2	12.0	.....	.....
	3	cross	7	.....	43.6	39.6
	3	self		1.0	.8	.9
	3	insect		6.5	5.8	1.6
II	4	cross	5	.....	6.3	.....
	4	cross	6	13.1	14.1	.....
	4	self		.4	.4	.....
	4	insect		.6	.4	.3
	5	cross	6	.....	15.4	.....
	5	cross	4	.....	3.3	.....
	5	self		.....	.6	.....
	5	insect		2.3	1.1	.4
III	6	cross	7	.....	7.6	7.1
	6	self		.....	0.0	.4
	6	insect		5.7	6.6	1.7
	7	cross	1	.....	.....	8.6
	7	cross	2	.....	.....	35.1
	7	cross	3	.....	.....	30.1
	7	cross	6	.....	.....	12.5
	7	self		.....	.....	.9
	7	insect		6.9	7.5	2.1

[1]Since there are two seeds per samara, multiplying the percentage values by 2 gives the number of filled seeds per 100 samaras.

[2]Cross- and self-pollinations were controlled; insect pollinations were uncontrolled.

stigmas. Near the end of the second day after flowering the milky-white mucus of the stigmas turns brown and the stigmas begin to shrivel. Apparently the stigmas are receptive to pollen for only about 12 to 24 daylight hours.

During flowering many insects were found on and around the open flowers. The most common were thrips, beetles, flies, and bees[2]. The common flower thrip *Frankliniella tritici* (Fitch) was found in all open flowers and in most unopened buds when the petals were loose. Thrips are probably of no significance in cross-pollination. Several species of beetles were found in and around the flowers. *Melyrodes cribrata* Le C., *Osypha varians* Le C., and two species of the family *Melachiidae* were very common. We also found seven other species of beetles that were either in the flowers or flying around them. Some of the beetles appear to be important pollinators. There were three species of bees of the families *Halictidae* and *Andrenidae* and three species of ants. The bees are important pollinators and there was much pollen on their legs. Many of the flowers contained ants that may have been tending aphids, but the ants are certainly of no significance in cross-pollination.

Cross-pollination of yellow-poplar flowers by insects seems to be relatively inefficient. Our observations and those of Wright (*17*) indicate the number of insects appeared sufficient to insure pollination. Even so, a great deal of selfing seemed to take place. Pollinating insects do not travel far and it is probably rare for trees 1 mile or more apart to be cross-pollinated. If compatible trees are adjacent the chances for effective pollination are, of course, increased. However, adjacent trees are likely to be closely related and have lower compatibilities than trees 1 mile or more apart. Also, our observations indicate that each insect comes in contact with only a few of the approximately 80 stigmas of each flower and this tends to reduce the chances of all stigmas being effectively pollinated. Controlled cross-pollinations among trees of the same stand usually result in a higher percentage of filled seed than uncontrolled insect pollinations (table 1). This is additional evidence that insect pollinations do not result in effective pollination of all stigmas.

Our observations also indicate that weather influences insect activity and may partially account for the observed annual variations in seed viability (*11*). All of the sample trees produced a smaller percentage of filled seed from insect pollinations in 1960 than in 1958 and 1959 (table 1). Our records and those of the local weather stations show there were more rainy and cool, cloudy days during May 1960 than in May 1958 and in May 1959. Since many insects are not as active on such days as on clear, warm days, it is likely that reduced insect activity resulted in fewer cross-pollinations. Although this is only one observation, it seems logical that the number of filled seed is related to the number of clear, warm days during the flowering period.

Fertilization

We did not observe fertilization, but we have reason to believe the embryo and endosperm originated as a result of fertilization. We expected fusion to occur between the nucleus of a sperm cell and the nucleus of the egg cell, and between another nucleus of another sperm cell and the two polar nuclei. The former would result in the diploid nucleus of the zygote from which the embryo develops, whereas the latter would produce the triploid endosperm. Only indirect evidence that this occurred was observable. We found undifferentiated-but-detectable, singly occurring embryos near the micropylar end of the developing seed and the embryo was always enclosed by endosperm cells with large nuclei. These observations and the fact that the embryo sacs are normal are good indications that fertilization occurred. Also the large nuclei of the endosperm cells indicate the triploid condition. This is good evidence that the polar nuclei united with a gamete.

[2]Dr. John C. Downey, Entomologist of Southern Illinois University, assisted in collecting and identifying the insects. Dr. Lewis J. Stannard, Illinois Natural History Survey, identified the flower thrip.

Development of Viable Seeds

Guard (*6*) has reported on embryo and endosperm development, and our findings agree essentially with his. There is apparently a delay in development of the embryo after the first division of the zygote, although the endosperm is slowly developing. The ovule increases greatly in size shortly after the period of pollination.

By the first week in July, in those seeds that were found with embryos, the endosperm was well developed with but a small amount of nucellus left. However, the embryo was less than 1/7 millimeter in diameter and was globular in shape on the end of its suspensor and without evidence of any localized differentiation (fig. 7).

By August the cellular endosperm was well developed, and the single embryo was about 1 millimeter in length with the hypocotyl slightly longer than the two cotyledons, and the epicotyl poorly developed.

During the last week of October, the endosperm again appeared cellular and whitish in color, and by this time occupied most of the area within the seed coats, the nucellus being much reduced and brownish. The single embryo in its usual position near the micropylar end measured approximately 1½ millimeters in length, with the hypocotyl still slightly longer than the cotyledons and with the epicotyl still only slightly developed (fig. 8). The average length of such seeds was about 5½ millimeters.

In the few instances observed with the more unusual condition of two filled seeds each with an embryo and endosperm in one samara, both seeds were approximately the same length (5½ millimeters) and in both the embryos were of the same size and proportions mentioned.

Development of Non-Viable Seed

Non-viable seeds develop from normal, well-developed ovules that contain fully formed embryo sacs with apparently functional eggs and fusion nuclei. When an egg is not fertilized, the embryo sac disintegrates and leaves a cavity within the

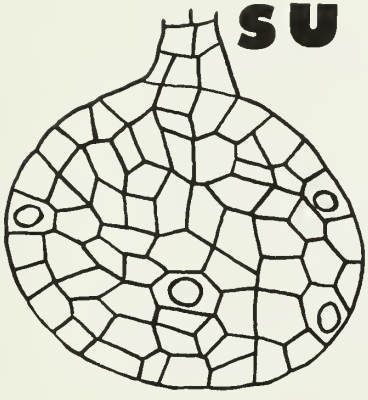

FIGURE 7. — *Drawing of an embryo during the early spherical stage of development. The embryo is attached to the ovule by a thick suspensor* (su) *and does not differentiate tissues until late July or early August. However, the endosperm tissue is well developed and fills most of the seed coat.*

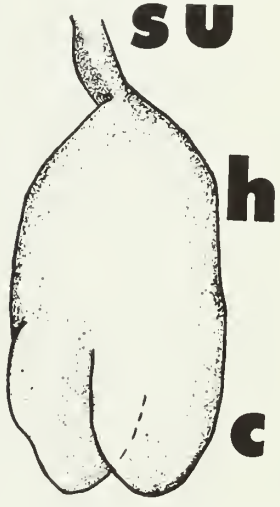

FIGURE 8. — *Drawing of a mature embryo in late October. The embryo, about 1½ millimeters in length, is still attached to the suspensor* (su) *and has two cotyledons* (c) *and a hypocotyl* (h).

nucellus. However, the integuments continue to grow and eventually form a full-sized and normal seed coat. The nucellus continues to grow and fills the developing seed coat in June and July (fig. 3). In late July and August, rarely in June, the nucellar tissue turns brown and begins to disintegrate. When the cones mature in late September and October, most non-viable seeds consist of fully formed but hollow seed coats that contain browned, dried remnants of the nucellus.

The nucellar tissue that fills unfertilized and non-viable seed can be distinguished from the endosperm tissue of fertilized seed: Cells of the nucellar tissue contain normal-sized nuclei while cells of the endosperm contain very large nuclei. Also, in the development of viable seed both tissues are present, the endosperm developing at the expense of the nucellus.

In the fall a few non-viable seed are partially filled with a cottony, white substance. This material is composed of large empty cells and occurs inside the browned nucellus. The cells are about the same size as endosperm cells in viable seed. This may be degenerated endosperm and may indicate, as suggested by Guard and Wean (7), "the remnant of a seed that has failed in a late stage of development." Less than 1 percent of the samaras contained seeds with this cottony, white substance. If this material is degenerated endosperm, it means a few seed are non-viable because of deterioration after fertilization.

Parthenocarpy

Parthenocarpy is the rule rather than the exception for yellow-poplar. Full-sized cones and samaras develop from unpollinated flowers but the seeds are empty. There is no difference in the external appearance of cones from pollinated and unpollinated flowers, and there is no certain way, externally, to detect samaras with filled seed from those with empty seed.

We emasculated and bagged, but did not pollinate, 100 flowers. Many cones were lost in a windstorm, but 56 developed to maturity and were collected in September. No seedlings germinated from 40 of these cones and no embryos were found in sectioned cones. The seed coats developed normally and were filled with nucellus until July or August. At this time the nucellus disintegrated in the same way as in non-viable seed of fertilized flowers.

DISCUSSION AND APPLICATION OF RESULTS

Previous studies have shown a wide variation in the viability of seed among yellow-poplar trees but viability has not been related to trees of different diameters, to trees in open or closed stands, or to site fertility (7). A few observations have indicated a difference in seed viability due to the position of the cone on the tree or position of the seed in the cone, but these differences have been small and inconsistent. Our results do not disagree with any of these observations. Since the percentage of viable seeds is determined by a number of chance occurrences that result in effective pollination of some stigmas, it is logical to expect a wide range in the viability of seed among yellow-poplar trees. However, the most important factor is the occurrence of self- and cross-incompatibilities.

Self- and cross-incompatibilities in plants have been known for many years. They are known to occur in more than 800 species in 66 plant families among the angiosperms. Practically all are insect pollinated and have copious stigmatic fluid. Incompatibilities are physiological. The inhibiting effects are caused by chemical antagonisms between the diploid tissue of the pistil and the haploid tissue of the pollen. They are, however, controlled by heredity and are usually determined by multiple alleles which segregate at meiosis. The particular allele carried by the pollen grain most frequently determines incompatibility (*1, 3, 4, 9*).

Two types of incompatibility are most common among angiosperms (*1*). In one form inhibition commonly occurs at the stigmatic surface so as to inhibit pollen germination or to drastically curtail pollen

tube growth. This form of inhibition is usually complete and is probably not the form that occurs in yellow-poplar. The other common form of inhibition occurs at some stage during pollen tube growth in the pistil. During growth the pollen tube may burst before it reaches the ovule, it may merely stop growing, or it may grow so slowly that another more compatible tube reaches the ovule first. When pollen tubes with different alleles grow through the style at different rates, inhibition is not complete; and some self-fertilization may occur if cross-pollination is not effected during the receptive period of the stigma.

In yellow-poplar partial incompatibility could be caused by different rates of growth of pollen tubes associated with different alleles. Our observations and other studies (*2, 17*) show that some self-fertilization does occur, that pollen from distant trees is usually more effective than that from nearby trees; and that, if fertilization occurs, normal, viable seed will develop. Different rates of growth of pollen tubes from different sources could account for this situation.

Incompatibility in yellow-poplar gives rise to many practical problems for the forest geneticist. Since we cannot mass propagate yellow-poplar from cuttings, superior genotypes must be propagated by grafting or by seed. Grafting techniques for this species would probably be too expensive for forest plantings, but large numbers of seed could be produced in seed orchards.

Because of incompatibilities, however, it may be difficult to produce large numbers of yellow-poplar seed of superior genotypes. Since some selfing does occur, homozygous seed could be produced but yields would be low and the cost would be high. For the production of heterozygous seed, the level of incompatibility of the crosses would have to be balanced against the percentage of seed with superior genotypes. Ideally, we would like to have two or more highly compatible parents that produced seed with a high percentage of superior genotypes.

The presence of incompatibilities may mean difficulties in breeding yellow-poplar for desirable characteristics. If genes for a desirable characteristic are linked with genes for incompatibility, then only a portion of all possible gene combinations can be obtained in the offspring. Certain gametes will not occur in the offspring and the genetic ratios will be distorted. This situation should be considered in all yellow-poplar breeding studies.

In the establishment of a yellow-poplar seed orchard the major objective is to pollinate every stigma with compatible pollen from superior genotypes. Hand pollinating would be expensive. Some form of insect pollination may be cheapest and most effective. Since insects apparently travel among flowers of the same tree more frequently than among different trees, alternate planting or grafting of compatible trees would not completely solve the problem. An unknown percentage of the seed would still result from self-pollination, the yield of seed might still be relatively low, and an unknown percentage of the seed would have inferior genotypes.

One solution may be to use hives of bees in the seed orchard. If compatible pollen is placed on the bees as they leave the hive, then most flowers would be cross-pollinated. This should increase the percentage of seed with superior genotypes and increase the yield of seed.

It seems certain that yellow-poplar trees are not freely interbreeding under natural conditions and there is a low rate of gene interchange among stands. Most yellow-poplar samaras fall within 600 feet of the parent tree and it is probably rare for a viable seed to be blown a mile (*5*). Pollinating insects do not travel far and it is probably rare for insect pollination to occur between trees a mile apart. Because of this, seeds for nurseries should be collected from a number of trees located more than a mile apart. This will help to increase the genetic variation among seedlings used to establish plantations and increase the probability of highly compatible trees occurring near each other in the plantations. Also, increasing the

genetic variation would increase the chances of some of the seedlings being adapted to the planting site.

SUMMARY

Most non-viability in yellow-poplar seed is due to a lack of fertilization which is caused by ineffective pollination during the limited receptive period of the stigma. Ineffective pollination is caused by self- and cross-incompatibility of pollen and limited cross-pollination.

We found that the ovules, embryo sacs, and egg cells developed as in most seed plants; we found no irregularities in these processes that would prohibit fertilization or later development of the embryo or endosperm. The fact that these processes are normal eliminates many possible reasons for non-viable seed. The formation of pollen appeared normal and regular. The tube nucleus and the two sperm cells were observed to be structurally normal. Fertilization was not observed but indirect evidence indicates this process is normal.

When there is no effective pollination, the embryo sac disintegrates and leaves a cavity in the nucellus. The nucellus persists into late July and August, finally appearing macroscopically as shriveled, disintegrated tissue. This results in an empty seed consisting only of a full-sized seed coat and the remnants of the nucellus. Although lack of fertilization accounts for the low viability of most yellow-poplar seed, a few seed (less than 1 percent), may deteriorate after fertilization. The cottony, white substance sometimes found in seed coats in late summer may be degenerated endosperm.

No chromosomal irregularities were noted in any of the preparations. This eliminated another possible reason for non-viable seeds. The basic chromosome number for all trees investigated was 19.

During flowering the stigmatic surface of the pistil usually turns brown the second day after the flower opens and it is doubtful that pollination is effective after the second or third day. Since the flowers are insect pollinated, this is a relatively short time for pollination to occur with compatible pollen. This tends further to reduce the number of viable seed.

We found no evidence for embryos of possible maternal origin. Fifty-six emasculated, unpollinated flowers produced over 5,000 mature carpels but no viable seed.

Parthenocarpy is the rule rather than the exception for yellow-poplar. There is no difference in the external appearance of cones that develop from pollinated and unpollinated flowers. There is no certain way, externally, to detect samaras with filled seed from those with empty seed. And, samara size is no indication that the seed is filled or not.

At the base of each cone are 10 to 14 sterile samaras that have no ovules and no stigmas. Above these sterile samaras, position on the cone is no indication of whether or not a samara has filled or empty seed.

Controlled cross-pollinations usually resulted in higher percentages of filled seed than controlled self- and uncontrolled insect pollinations, and crosses among widely separated trees usually resulted in a larger number of filled seed than crosses among trees of the same stand.

Uncontrolled insect pollinations do not result in effective pollination of all stigmas. The number of insects appeared sufficient to insure pollination but a great deal of selfing seemed to take place.

Annual variations in the weather influence insect activity and may partially account for the observed annual variations in seed viability.

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